Methods: Fat conditioned medium (FCM) was made by culturing small pieces of infrapatellar fat in medium for 24 hours. The fat was obtained from osteoarthritic knees during total knee replacement. P2 synoviocytes, isolated from synovium obtained during total knee replacement, were seeded in high density and cultured in 9 different batches of FCM, representing different infrapatellar fat pad donors. Synoviocytes were also cultured in 10 ng/ml TGFβ1 as positive control. After 1 day, gene expression of myofibroblast markers PLOD2 (a cross-linking enzyme), α-smooth muscle actin (αSMA) and collagen type I was determined, after 4 days collagen content was measured. Furthermore, effects of FCM on proliferation (d1, 4, 5) and migration of the synoviocytes (scratch-wound assay, after 16 hours) were analyzed. Fat was also processed for immunodetection of CD68 (macrophages), CD86 (classically activated macrophages) and CD206 (alternatively activated macrophages).
Results: Fat Conditioned Medium induced synoviocytes to produce significantly more collagen (Figure 1) and express more PLOD2 (Figure 2) than when cultured in control medium. No clear effects were seen on collagen type I and αSMA gene expression. Synoviocytes cultured in FCM had a higher proliferation rate and migrated faster. Large numbers of CD68+ and CD206+ cells were present in the fat tissue. CD86+ cells were present in lower numbers.

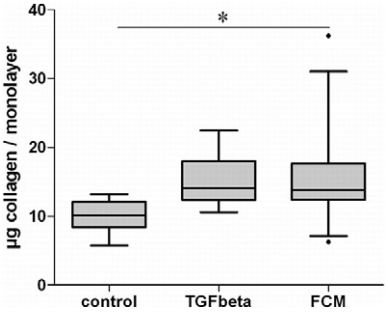

Figure 1. Collagen production by synoviocytes in monolayer. Average effect of 9 different batches of FCM is shown. *Indicates $p<0.05$.

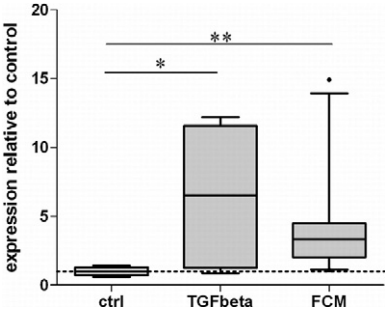

Figure 2. PLOD2 gene expression by synoviocytes in monolayer. Average effect of 9 different batches of FCM is shown. *Indicates $p<0.05$, **indicates $p<0.01$.

Conclusions: In conclusion, infrapatellar fat produces factors that can contribute to the development of synovial fibrosis by increasing collagen production, PLOD2 gene expression, cell proliferation and cell migration, all characteristics of a fibrotic process. A possible source for these factors could be the many alternatively activated (or wound healing) macrophages that were present in the fat tissue, since these cells are thought to be involved in tissue repair.

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MODULATION OF HYALURONIC ACID SYNTHESIS BY COLLAGEN HYDROLYSATES IN RABBIT SYNOVIOCYTES (HIG-82)

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Purpose: Collagen hydrolysate (CH) has been used as one of the functional foods for joint health. Some clinical studies have shown that oral ingestion of CH exerted its beneficial effect on OA symptoms. However, the efficacy of collagen-derived peptides has not been tested in clinical studies. So the therapeutic mechanisms remain unclear. There are several types of hydroxyproline (Hyp) -containing peptides in human blood after orally administered CH. Therefore we assessed whether CH-derived peptides and amino acids would influence hyaluronan (HA) synthesis in synoviocytes.
Methods: Rabbit synovial fibroblasts (HIG-82) were cultured in F-12 medium with 10%FBS. After 2 days, culture medium was replaced with serum free F-12 medium containing Gly-Pro-Hyp, Pro-Hyp, Hyp (50μg/ml) and incubated for 24h (RNA extraction) or 48h (HA assay). RT-PCR was performed to determine mRNA levels of hyaluronan synthases (HAS-1 and HAS-2). Culture medium was harvested for HA assay. HA content was measured by cellulose acetate membrane electrophoresis. Molecular weight of HA was estimated by agarose gel electrophoresis.
Results: Gly-Pro-Hyp and Pro-Hyp significantly up-regulated HAS-1 mRNA level in HIG-82 cells. Hyp treatment depressed markedly HAS-2 mRNA expression. But the amount of HA in the culture medium was not changed after the treatment of Gly-Pro-Hyp and Hyp. Pro-Hyp significantly stimulated the secretion of HA. The concentration of HA with a molecular weight greater than 700 kDa in the medium was higher in the presence of Hyp
Conclusions: Collagen hydrolysate-derived peptides enhanced HA synthesis in synoviocytes. This phenomenon is associated with increase of HAS-1 mRNA expression. In contrast, amino acid Hyp depressed HAS-2 mRNA expression. But HA content in the medium was not changed, and the molecular weight of HA significantly increased after Hyp treatment. These findings suggest that oral ingestion of CH may exert its beneficial effect on OA symptoms through increased high molecular weight-HA production into the synovial fluid.

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THE REGULATION OF THE ADAMTS4 AND ADAMTS5 AGGRECANASES IN OSTEOARTHRITIS

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Purpose: To investigate the regulation of the ADAMTS4 and ADAMTS5 aggrecanases in human osteoarthritis (OA) synovium, with regard to cytokine stimulation with IL-1 and/or TNFalpha, and also the degree of NFkappaB dependence.
Methods: To investigate the role of TNFalpha and IL-1 in driving ADAMTS4 and ADAMTS5 expression in the human OA synovium, we used a model of cultures of synovial cells from digested human OA synovium. It was possible to effectively and specifically neutralize the endogenous production of IL-1beta and/or TNFalpha from the OA synovial macrophages. Cultures were either left untreated, incubated with the p75 TNF soluble receptor Ig fusion protein etanercept (Enbrel), incubated with a neutralizing anti-IL-1beta antibody, or incubated with a combination of Enbrel and anti-IL-1beta. After incubation for 48 h, cells were washed and RNA extracted using Tri-reagent, to be taken to RT-PCR analysis using oligonucleotide primers specific for ADAMTS4 and ADAMTS5, with GAPDH used for comparison of gene expression.
The experiments with regard to the degree of NFkappaB dependence of ADAMTS4 and ADAMTS5 made use of outgrown human OA synovial fibroblasts. Treatment with IL-1beta or TNFalpha, but not treatment with phorbol ester, resulted in upregulation of ADAMTS4, whereas ADAMTS5 was unaffected by these stimuli. In this model, it was possible to use adenoviral gene transfer of the endogenous inhibitor IkappaBalpha to specifically inhibit NFkappaB, without affecting other intracellular signalling pathways, or inducing significant apoptosis.
Results: In OA synovial cell cocultures, there was no effect of either Enbrel or the neutralizing anti-IL-1beta antibody on ADAMTS5 expression, nor was this aggrecanase at all affected by a combination of these treatments. Thus ADAMTS5 appears to be constitutive in OA synovial cells. In contrast, ADAMTS4 was significantly ($p<0.05$) inhibited by Enbrel, and more potently ($p<0.01$) inhibited by a combination of Enbrel and the neutralizing anti-IL-1beta antibody. This would indicate that in the human OA synovium, the upregulation of ADAMTS4 is dependent on TNFalpha and IL-1 produced by the synovial macrophages, whereas the level of ADAMTS5 is not changed by these cytokines.
In OA synovial fibroblasts, ADAMTS5 gene expression was not changed by adenoviral gene transfer of IkappaBalpha, irrespective of stimulus used. In contrast, ADAMTS4 induction by IL-1beta or TNFalpha was potently inhibited by NFkappaB downregulation.
Conclusions: Although both ADAMTS4 and ADAMTS5 cleave aggrecanase,